

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

Claim 1. (previously amended) A method of measuring the production of a secreted target analyte of interest in a human or animal, comprising the steps of:

- a. injecting the human or animal with an amount of labeled neutralizing targeting moiety, wherein the targeting moiety binds specifically to the target analyte, and wherein the targeting moiety is injected in sufficient quantity that a measurable fraction of target analyte is bound by the labeled neutralizing targeting moiety;
- b. allowing the targeting moiety to circulate through the injected human or animal for a defined period of time sufficient to bind to the target analyte of interest and form a targeting moiety:target analyte conjugate wherein the formation of the targeting moiety:target analyte conjugate decreases the clearing rate of the target analyte;
- c. obtaining a sample of blood from the human or animal after the defined period of time;
- d. combining the sample of blood with a capture moiety wherein the capture moiety binds specifically to the targeting moiety:target analyte conjugate in order to form an assay mixture;
- e. incubating the assay mixture of step d to allow the capture moiety to bind to the targeting moiety:target analyte conjugate and form targeting moiety:target analyte:capture moiety complexes in the assay mixture;
- f. removing any unbound and unconjugated targeting moiety and target analyte from the assay mixture;
- g. detecting the amount of labeled targeting moiety:target analyte:capture moiety complexes;

- h. wherein the amount of labeled targeting moiety:target analyte:capture moiety complexes detected in step (g) provides a measure of the production of secreted target analyte in the sample during the defined period of time; and
- i. wherein the secreted target analyte is a secreted cytokine, secreted peptide or secreted protein hormone.

Claim 2 (cancelled)

Claim 3 (cancelled)

Claim 4. (previously amended) The method of claim 14, wherein the target analyte is a cytokine.

Claim 5. (original) The method of claim 4, wherein the cytokine is selected from the group consisting of interleukins, interferons chemokines, growth factors, colony stimulating factors, lymphokines, lymphotoxins, and tumor necrosis factors.

Claim 6. (previously amended) The method of claim 4, wherein the cytokine is selected from the group consisting of interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, interleukin-14, interleukin-15, interleukin-16, interleukin-17, interleukin-18,, interferon-alpha, interferon-beta, interferon-gamma, lymphotoxin, tumor necrosis factor-alpha, transforming growth factor (TGF)-beta, granulocyte macrophage-colony stimulating factor (GM-CSF), nerve growth factor (NGF), and epidermal growth factor (EGF).

Claim 7. (previously amended) The method of claim 1, wherein the blood is selected from the group consisting of whole blood, serum and plasma.

Claim 8. (previously amended) The method of claim 1, wherein the targeting moiety is selected from the group consisting of antibodies, soluble receptors, and recombinant molecules with binding sites for the target analyte.

Claim 9. (previously amended) The method of claim 8, wherein the targeting moiety is a monoclonal antibody.

Claim 10. (original) The method of claim 1, wherein the capture moiety is an antibody.

Claim 11. (original) The method of claim 10, wherein the antibody is a polyclonal antibody which recognizes many epitopes on the target analyte.

Claim 12. (previously amended) The method of claim 9, wherein the targeting moiety is detectably labeled, wherein the label is selected from the group consisting of radioisotopes, affinity labels, enzymatic labels, and fluorescent labels.

Claim 13. (previously amended) The method of claim 1, wherein the targeting moiety is labeled with a small molecule hapten and wherein the method further comprises the step of binding the small molecule hapten to a binding partner which is conjugated to an enzyme.

Claim 14. (previously amended) The method of claim 1, wherein the defined period of time is from about 1 hour to about 72 hours.

Claim 15. (previously amended) The method of claim 13, wherein the hapten is biotin.

Claim 16. (original) The method of claim 13, wherein the enzyme-conjugated binding partner is selected from the group consisting of streptavidin, anti-biotin antibody, anti-hapten antibody, and anti-immunoglobulin antibody.

Claim 17. (original) The method of claim 13, wherein the enzyme is selected from the group consisting of alkaline phosphatase, glucose oxidase, beta -galactosidase, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, asparaginase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

Claim 18. (original) The method of claim 12, wherein the targeting moiety is labeled by linking to a fluorescent labeling compound.

Claim 19. (original) The method of claim 18, wherein the fluorescent labeling compound is selected from the group consisting of fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Claim 20. (previously amended) The method of claim 8, wherein the labeled targeting moiety comprises first and second members of a complimentary ligand/anti-ligand pair, wherein the first member of the complimentary ligand/anti-ligand pair is injected as the targeting moiety in step (a); wherein the second member of the complimentary ligand/anti-ligand pair is a detectable binding partner to the first member; and wherein the method further comprises the steps of (I) contacting the assay mixture after step (e) and before step (f) with the second member of the complimentary ligand/anti-ligand pair to allow binding of the first and second members; (II) removing any unbound second member; (III) detecting the amount of bound second member; and (IV) correlating the detected amount to the amount of targeting moiety:target analyte:capture moiety complexes in the assay mixture; wherein the amount of targeting moiety:target analyte:capture moiety complexes detected provides a measure of the production of secreted target analyte during the defined period of time.

Claim 21. (previously amended) The method of claim 20, wherein the first member of the complimentary ligand/anti-ligand pair is a monoclonal antibody.

Claim 22. (previously amended) The method of claim 21, wherein the capture moiety is an antibody.

Claim 23. (previously amended) The method of claim 22, wherein the capture moiety is a polyclonal antibody.

Claim 24. (cancelled)

Claim 25. (previously amended) The method of claim 20, wherein the targeting moiety:target analyte:capture moiety complexes are detected by radioimmunoassay.

Claim 26. (previously amended) The method of claim 20, wherein the second member of the complimentary ligand/anti-ligand pair is detectably labeled by an enzymatic label.

Claim 27. (original) The method of claim 26, wherein the label is a small molecule hapten.

Claim 28. (original) The method of claim 27, wherein the hapten is biotin.

Claim 29. (previously amended) The method of claim 26, wherein the second member of the complimentary ligand/anti-ligand pair is selected from the group consisting of streptavidin, anti- biotin antibody, anti-hapten antibody, and anti-immunoglobulin antibody.

Claim 30. (original) The method of claim 26, wherein the enzyme is selected from the group consisting of alkaline phosphatase, glucose oxidase, beta-galactosidase, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, asparaginase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

Claim 31. (previously amended) The method of claim 20, wherein the second member of the complimentary ligand/anti-ligand pair is labeled with a fluorescent label.

Claim 32. (original) The method of claim 31, wherein the fluorescent labeling compound is selected from the group consisting of fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Claim 33. (previously amended) The method of claim 1 or 20, wherein the capture moiety is immobilized on a solid phase support.

Claim 34-42. (canceled)